WATER SOLUBLE VITAMIN REQUIREMENTS OF SILVER SALMON

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WATER-SOLUBLE VITAMIN REQUIREMENTS OF SILVER SALMON

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ABSTRACT

Qualitative vitamin requirements of silver salmon (Oncorhynchus kisutch) were determined by feeding groups of fingerlings a complete vitamin test diet (casein 54, gelatin 15, corn oil 7, cod liver oil 2, dextrin 8, minerals 4, methionine 1.0, tryptophan 0.5, celluflour plus crystalline vitamins 8.5) as the control diet; and deleting one water-soluble vitamin at a some from the ration for the respective deficient lots. A 16-week feeding period resulted in vitamin deficiency syndromes appearing in thiamine, pyridoxine, folic acid, biotin, pantothenic acid, inositol and choline deficient groups. Nicotinic acid, riboflavin and cyanocobalamine deficient lots gave inconclusive results, and under experimental conditions used no deficiency syndromes were observed for fish without ascorbic acid in the diet.

WATER-SOLUBLE VITAMIN REQUIREMENTS OF SILVER SALMON

Good fish husbandry requires the use of diets which provide the nutrients essential for normal growth of the fish. A lot of "practical" information has been accumulated by fish culturists on food for silver salmon (Oncorhynchus kisutch), but there is little published data on specific nutritional requirements for this species. Nutritional investigations with other fish, rainbow trout (Salmo gairdneri), chinook salmon (O. tshawytscha) and sunfishes (Lepomis), have yielded more concrete data on qualitative and quantitative needs for growth and metabolism. Vitamin requirements (Phillips et al. 1945, 1947, 1949, 1950; McLaren et al. 1947; Wolf 1951; Halver 1957a), general protein requirements (Tunison et al. 1942, 1943; Gerking 1952; De Long, Halver and Mertz 1957) and amino acid requirements (Halver 1957b; Halver, DeLong and Mertz 1957, 1958) have been described which can serve as a basis for formulating test diets and experimental rations for determining the basic nutritional requirements of silver salmon. If the general requirements for all nutrients is in the same general range as that found for trout and chinook salmon, and if the same experimental techniques can be applied to studies with silver salmon, then it should be possible to use existing test diets to develop specific nutritional deficiency syndromes in silver salmon and determine the spectrum of the water-soluble vitamin requirements for this species.

As a preliminary logical step, silver salmon were tested with the same vitamin-test diet used for qualitative vitamin requirement studies with chinook salmon (Halver 1957a) and which also maintained rainbow trout for at least one reproductive cycle (Halver and Coates 1957). Since silver salmon fingerlings grew when fed this diet as sole ration, it was then possible to delete one vitamin at a time from the complete vitamin mixture in the diet, to feed these specific water-soluble vitamin deficient diets to various individual lots of fingerlings, to describe the specific vitamin-deficiency syndromes as they occurred in each respective lot of fish, and to determine which water-soluble vitamins were required for growth and fresh water survival of silver salmon.

EXPERIMENTAL

The complete vitamin-test diet was formulated from the materials listed in table 1. The procedure used was the same as that described in detail for the preparation of diets used previously to induce vitamin deficiency syndromes in chinook salmon (Halver 1957a). Prior to mixing the diet, the crystalline vitamin supplement, the amino acid supplement and the alpha-cellulose flour were mixed for two hours in a ball mill and then stored at 5° to 10° C., until used. To ensure more accurate and reproducible weights, sufficient alpha-cellulose flour, amino acids, and vitamins for at least 4 kg of diet were mixed at one time. The mineral mixture was also ground for two hours in a ball mill and stored in the cold in a tight container until needed.

To prepare 400 gm of mixed diet containing 25 percent solids and 75 percent water, 15 gm of purified gelatin were added to 300 ml of water and heated on a hot plate until the temperature rose to 60° to 70° C. After the gelatin had liquefied, the container was removed from the hot plate, placed in a mechanical mixer and stirred at medium speed with a dough hook until the temperature dropped to 40° to 50° C. Then 54 gm vitamin test casein, 7 gm purified corn oil, 8 gm white dextrin and 4 gm mineral mixture were added and thoroughly blended. Finally, the cod liver oil and the alpha-cellulose flour containing the vitamin mixture and the amino acid supplement were added and stirred until a homogeneous mass was obtained (30° to 35° C.). For convenience in feeding, the mixture was poured into ice cube containers, hardened in a refrigerator at 10° C., and stored in screw-top glass jars at 5° to 10° C. until used.

Approximately 7,000 silver salmon from the 1956 brood were fed two weeks on a complete vitamin test diet containing one-fourth the normal amount of vitamin mix at the Washington State

TABLE 1.--Composition of the vitamin-test diet for silver salmon.

Main mixture	Parts	Mineral mixture	Amount	Vitamin supplement	Amount[1/]
					mg
Vitamin-free casein	54	U.S.P. XII Salt Mixture No. 2	100 gm	Thiamine-hydrochloride	5
Gelatin, purified	15	Aluminum chloride	15 mg	Riboflavin	20
Corn oil, purified	7	Zinc sulfate	300 mg	Pyridoxine hydrochloride	5
Cod liver oil	2	Cuprous chloride	10 mg	Nicotinic acid	75
White dextrin	8	Manganous sulfate	80 mg	Calcium pantothenate	50
Mineral mixture	4	Potassium iodide	15 mg	Inositol	200
DL-Methionine	1	Cobaltous chloride	100 mg	Biotin	0.5
L-Tryptophan	0.5			Folic acid	1.5
Alpha-cellulose flour + Vitamin supplement	8.5			Choline chloride	500
				Ascorbic acid	100
				Crystalline vitamin B_{12}	0.010
				Alpha-tocopherol	40
				Menadione	4

[1/] These amounts of individual vitamins were added per 100 gm of the main mixture.

Department of Fisheries Issaquah Hatchery. This vitamin level was fed in order to deplete any large storage of water-soluble vitamins by the fish. At the end of two weeks the fish were randomly divided into 13 groups and placed in standard screen-covered hatchery shallow troughs. Each group was fed a diet deficient in only one of the 11 water-soluble vitamins and the growth was compared with two control lots fed the complete vitamin-test diet. The fish were fed three times daily, six days weekly with no ration offered on Sundays. The food was presented to the fish by grating small cubes of the ration over the water in such a manner that the food presented was eaten in approximately one minute.

The fish were weighed biweekly according to a standardized experimental technique (Burrows, Robinson and Palmer, 1951; Halver 1957a) and a random sample of five fish was taken for subsequent histopathological analysis.

Each group was under close examination for the appearance of vitamin deficiency syndromes listed for fish (Halver 1957a; McLaren et al. 1947; Wolf 1951). Dead and moribund fish were removed, recorded daily and were examined microscopically for any indication of the presence of fish pathogens.

When the growth pattern of any of the test diets differed significantly from the control or when more than 20 percent of the population had died, the remaining population of the test group was divided, one-half fed the complete diet and the other half continued on the vitamin-deficient diet until the end of the experiment or the total loss of the population. If that portion of the population fed the complete diet recovered from the symptoms, it was then felt that the malady was directly due to the lack of the vitamin in question and that silver salmon required this vitamin.

The regular hatchery water supply from Issaquah Creek was used. The water to the individual trough was screened through a 64-mesh screened box to eliminate as much as possible the natural food that might come in through the water supply. Finer mesh screen was tried but this had a tendency to plug and overflow subsequently cutting off the water supply to the experimental fish.

Throughout the experiment many pathogenic diseases were apparent. The two encountered most often were "cold water disease" and "Octomitus". The effect of the pathogen certainly influenced the results, and it was impossible to evaluate growth and mortalities critically. Growth curves of the deficient groups were plotted however, and can be generally compared with the growth and mortality rates of the two control lots fed the complete vitamin-test diet (figs. 1 and 2).

RESULTS AND DISCUSSION

The two control groups gained weight consistently. There was little if any significant difference between the two. This group suffered approximately 50 percent loss over the 16-week period but all mortalities were heavily infected with Hexamita salmonis and/or myxobacteria. The greatest mortality occurred during the fourth week. A summary of the specific vitamin deficiency syndromes observed in the various lots of fish was tabulated in table 2.

The diet used as the control ration for this study contained approximately 70 percent protein. Subsequent work has indicated that about 50 percent protein might have been a more desirable level for rapid growth with fish living in this temperature range (DeLong, Halver, Mertz 1957). At the time the experiment started, however, this information had not yet been conclusively demonstrated and it was decided to use the complete vitamin-test diet which did produce near normal growth in chinook salmon for a time period sufficient for the development of water-soluble vitamin deficiency syndromes. For a similar reason, the protein component of the diet was supplemented with methionine and tryptophan even though some evidence had been accumulated that these amino acid supplements to this diet might not be needed for satisfactory growth. High levels of tryptophan would, in addition, probably interfere with the development of severe nicotinic acid deficiencies but since the tryptophan requirements of fish were not known, and this diet had grown chinook salmon, tryptophan was added.

The thiamine deficiency group compared favorably with the control groups for the first 12 weeks of the experiment. After 12 weeks

Figure 1 and 2:--Growth and mortality of vitamin-deficient silver salmon. Upper curves show growth of specific deficient lots and the control lots. Lower curves show biweekly mortality percentage of survivors. The junction points in growth and mortality curves represent division of the deficient groups into two sublots after the deficiency syndromes became apparent in a large portion of the population.

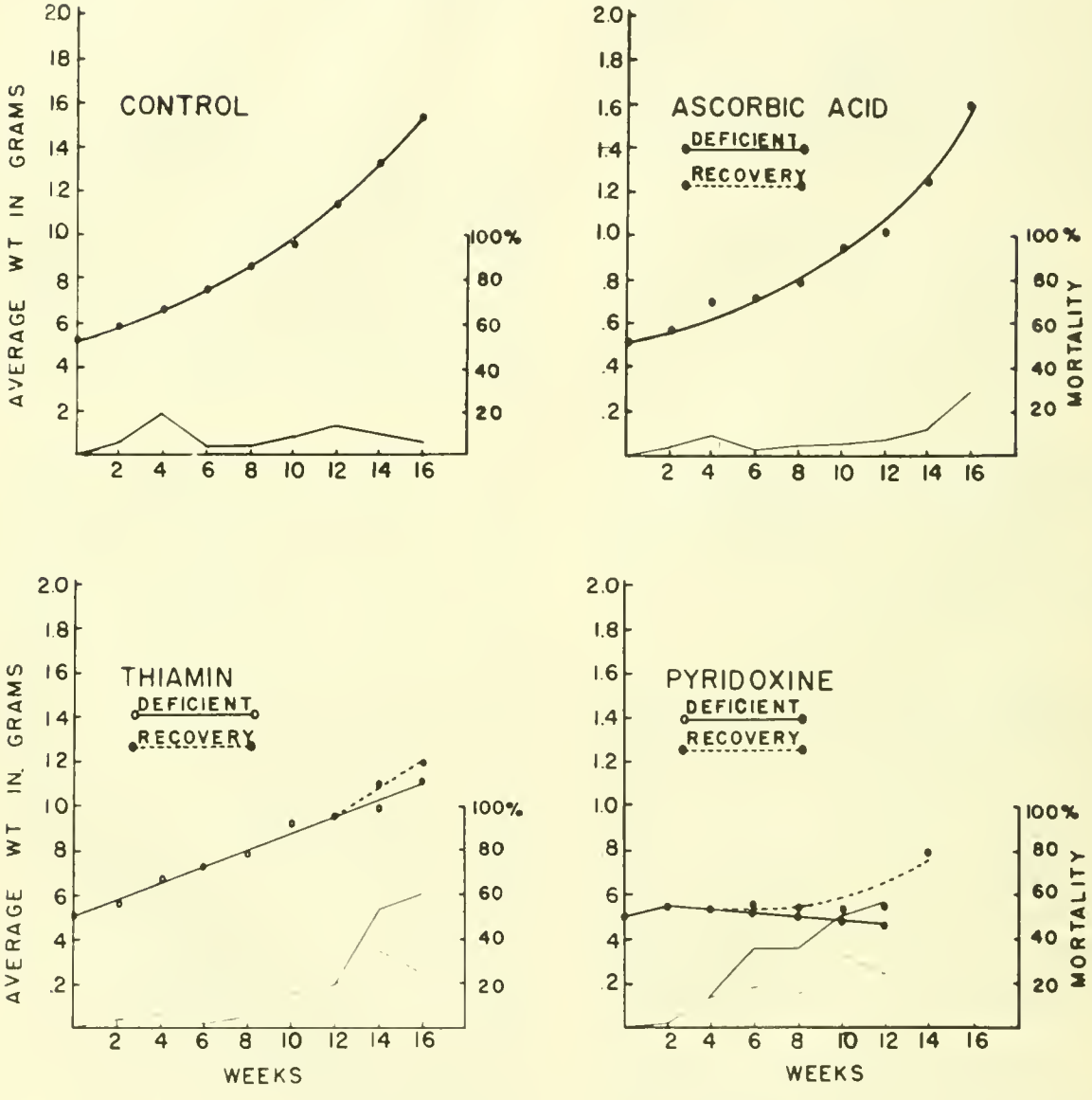

Figure 1

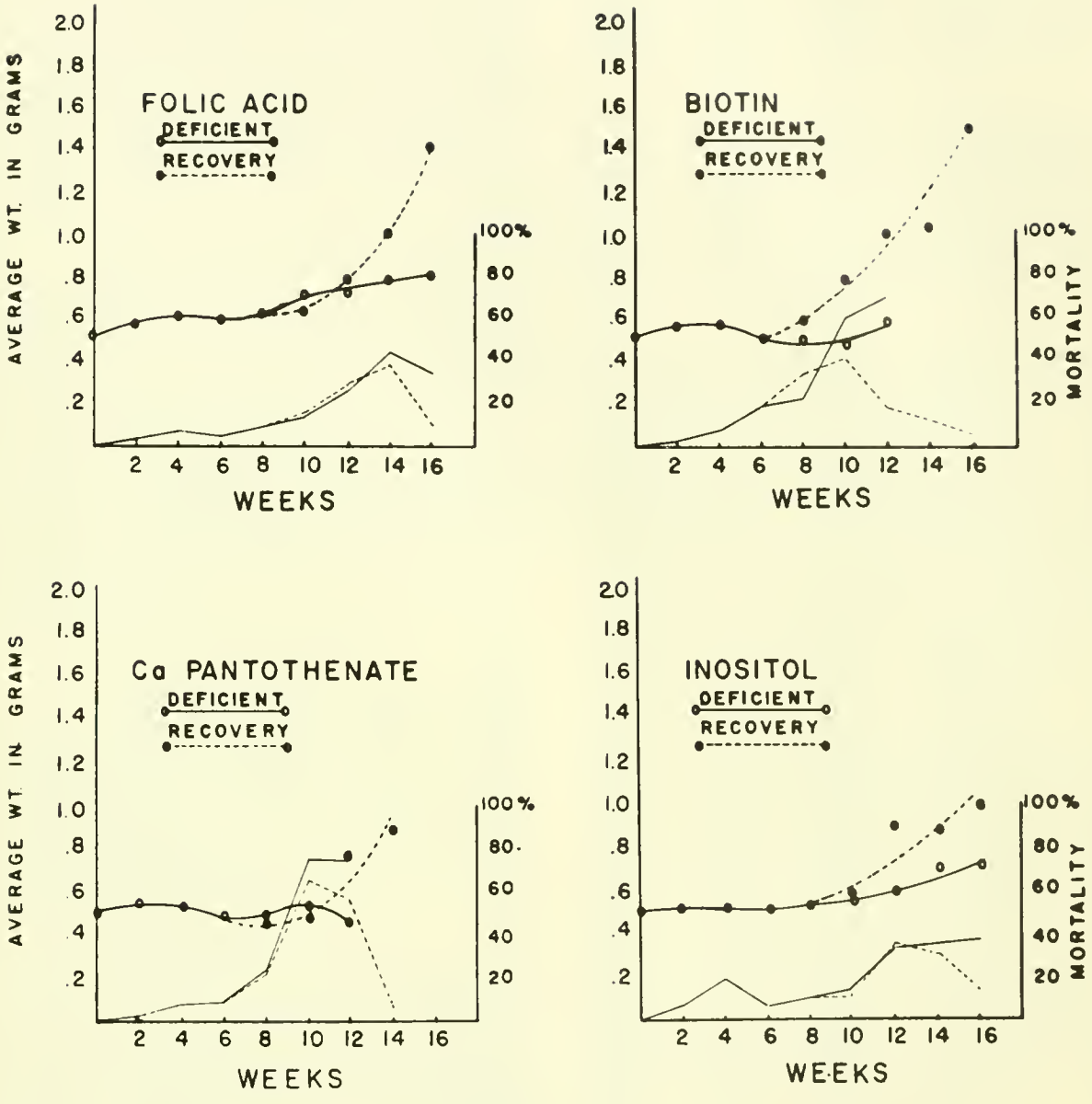
FOLIC ACID
DEFICIENT
RECOVERY
AVERAGE WT. IN GRAMS
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
100%
80
60
40
20
MORTALITY
2 4 6 8 10 12 14 16
WEEKS
BIOTIN
DEFICIENT
RECOVERY
Ca PANTOTHENATE
DEFICIENT
RECOVERY
INOSITOL
DEFICIENT
RECOVERY

Figure 2

TABLE 2.--Signs of avitaminosis in silver salmon fed vitamin-deficient diets.

Vitamin deficiency	Silver salmon	other fish [1, 2, 3]
Thiamine	Poor appetite; convulsions prior to death; instability and loss of equilibrium.	Poor appetite; muscle atrophy; convulsions in acute stage; instability and loss of equilibrium.
Pyridoxine	Nervous disorders; hyperirritability; poor appetite; indifference to light; post-mortem rigor mortis occurs rapidly.	Nervous disorders; hyperirritability; poor appetite; post-mortem rigor mortis occurs rapidly; rapid and gasping breathing; flexing of opercles.
Folic acid	Poor growth; loss of appetite.	Poor growth; loss of appetite; anemia; lethargy; dark coloration; megaloblastic erythropoesis.
Biotin	Poor growth, loss of appetite; contracted caudal fins.	Poor growth; loss of appetite; spastic convulsions; fragmentation of erythrocytes; muscle atrophy; "blue slime skin".
Pantothenic acid	Clubbed gills; prostration; loss of appetite; gills covered with exudate; sluggishness.	Clubbed gills; prostration; loss of appetite; necrosis and scarring; cellular atrophy; sluggishness; gills covered with exudate.
Inositol	Poor growth; loss of appetite.	Poor growth; loss of appetite; distended stomach; increased gastric emptying time.
Choline	Poor growth; poor food conversion.	Poor growth; poor food conversion; hemorrhagic kidney and intestines.
Ascorbic acid	No abnormal indication in growth, appetite, mortality.	No abnormal indication in growth, appetite, mortality.

[1] Halver 1957a.
[2] McLaren et al. 1947.
[3] Wolf 1951.

feeding gradually ceased and many emaciated fish or "pin heads" with a characteristic severe concave or "pinched" abdomen occurred. Some loss of ability to control the dorsal and pectoral fins was noted in moribund specimens. Locomotion consisted of a slow, rolling action and a general loss of equilibrium. The coloration of affected fish changed from a dark hue at the onset of the clinical manifestations of the deficiency to a more transparent condition when moribund or dead.

The pyridoxine deficiency syndrome manifested itself by loss of equilibrium and by rapid flashing and erratically skipping action along the surface of the water just prior to death. Upon death, these fish assumed a lateral crescent shape and were light in color. The characteristic blue color previously described in chinook salmon (Halver 1957a) was not noted. During the early stages of the malady, the appetite was fair but changed to a complete lack of feeding during the late stages of the experiment. Severely affected fish exhibited indifference to strong light in contrast to the controls' definite negative phototropism.

The folic acid group exhibited no clinical syndrome and periodic blood counts did not indicate any gross anemia. However, when this group was divided, the section receiving the complete diet indicated a definite benefit from the addition of folic acid by a rapid growth response and a significant increase in weight over the deficient lot.

The biotin deficiency syndrome was characterized by loss of appetite, emaciated bodies and the caudal fins were contracted to form a triangular point. Although the feeding habits of the deficient fish were retarded, the individuals seemed fairly active throughout the experiment. The recovery group regained normal appetite and weight quickly, and within 2 weeks these fish appeared normal. Mortalities, fairly consistent in other groups, were virtually non-existent in the biotin recovery group after replacement of the missing vitamin in the diet.

Pantothenic acid depleted fish had the characteristic clubbed gills previously noted by many investigators. As the syndrome started to appear, these fish gradually lost their appetite until they ceased feeding entirely near the end of the experiment. The recovery group exhibited a rapid positive response in appetite and growth as soon as the gill damage was repaired, and near the end of the experiment, was gaining rapidly.

The appetite and growth of the inositol deficient group was reduced, although a consistent gain in weight was noted. Upon replacement of the missing vitamin in the diet the recovery group showed a significant growth response and a corresponding reduction in mortality from that of the deficient lot.

The choline deficient fish fed actively throughout the experiment but failed to gain weight, resulting in emaciated fish in the entire population. When one-half the group was fed the complete diet, they immediately started to gain whereas the group continued on the choline deficiency continued to lose weight. The growth curve of the choline deficient group was not graphed because a general straight line relation existed from the start to the end of the experiment, and the mortality rate was consistently low throughout.

Disease decimated the niacin deficient group before any specific syndromes became apparent. This group was split at the end of 12 weeks but at the 16-week period there were no significant differences between the deficient and recovery groups. Since these fish were held indoors, the common "sunburn" symptom described by DeLong, Yasutake and Halver (1958) in chinook salmon and rainbow trout did not appear. Exposure of these fish to ultraviolet light in a more carefully controlled environment might have resulted in the appearance of a specific deficiency syndrome.

Similarly, the riboflavin deficient group did not exhibit any gross clinical symptoms. Growth continued parallel to the controlled lot throughout the course of the experiment but a slight clouding of the lenses of the eyes appeared in some of the moribund fish late in the experiment. Some food was probably introduced through the screens in the creek water supply and may have been sufficient to partially supplement the diet to an extent preventing severe clinical manifestations of riboflavin deficiency.

In the vitamin B_{12}-deficient lot, no deficiency syndromes were noted, even when this group was divided after 12 weeks on the depleted diet. Those fish receiving the complete test diet exhibited little difference from those fed the diet without vitamin B_{12}.

In the ascorbic acid deficient group, growth comparable with the controls was observed throughout the course of the experiment. The mortality rate paralleled closely that of the control groups at least for the first 14 weeks of feeding. The group as a whole seemed healthy and showed less disease incidence than any other group, except the controls, until the last 2 weeks of the experiment. During the last 2 weeks of feeding, most fish examined in all lots showed a high incidence of Hexamita, myxobacteria, or both.

The entire course of the experimental feeding period was beset with problems of Hexamita infestation, myxobacterial infection and natural-food problems. The water temperature gradually increased from a low of 54° F. to a high of 68° F. during the middle of the feeding period. Unfortunately, the temperature remained high (in the low 60's) until the 16-week feeding period was terminated. In the mornings the temperature was in the middle 50's, increasing from 7° to 10° F. during each day. Because of the severe disease incidence, repeated prophylactic treatments with pyridal mercuric acetate were given for one hour every two weeks starting at the sixth week of feeding. Undoubtedly, some adverse physiological effects were experienced by the fish which may have tended to obscure the appearance of specific vitamin deficiency syndromes.

These experiments show again the necessity of having a fish disease-free water supply for conducting critical nutrition experiments in order to prevent the disease symptoms from masking the appearance of the deficiency syndromes. The desirability of a more stable or controlled water temperature also manifested itself with the extreme variation in even daily temperatures reflecting severely on the feeding habits of the various groups of fish and on the calculation of the correct dietary intake for each lot. Perhaps more descriptive information of subclinical manifestations of the deficiency syndromes will be obtained upon completion of the histopathology investigations of the tissues of fish collected during the course of the experiment.

SUMMARY

A complete vitamin-test diet successfully used to induce specific water-soluble vitamin deficiencies in chinook salmon was fed to silver salmon for a 16-week feeding period. Deleting the water-soluble vitamins one at a time from this complete diet caused deficiency syndromes to appear. Under the experimental conditions used, some deficiency syndromes for thiamine, pyridoxine, folic acid, biotin, pantothenic acid, inositol, and choline were induced in silver salmon. Inconclusive results were obtained with niacin, riboflavin and vitamin B_{12} deficient diets. No deficiency syndromes were observed for the ascorbic acid deficient lot and since p-amino benzoic acid was not included in the vitamin mixture, it also was probably not required.

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